

Bio-inspired enzyme entrapment and cross-linking approaches as alternative tools for enzyme immobilization

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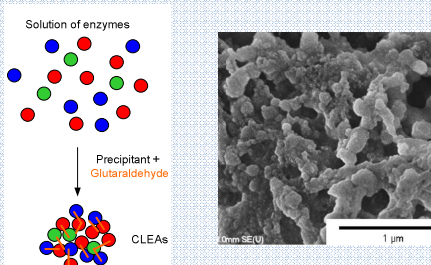
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OXIDATIVE ENZYMES Laccases, oxidases, peroxidases



Approach 1: Cross-linking enzyme aggregates (CLEAs) [1,2]

➤ **Biocatalyst:** Laccase from *C. polyzona*



➤ **Application:** Degradation of bisphenol A in a continuous membrane reactor

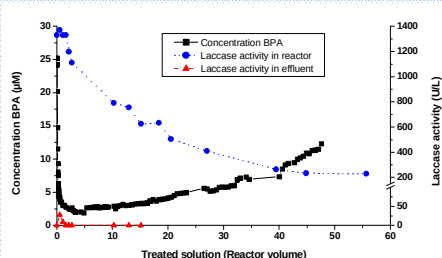
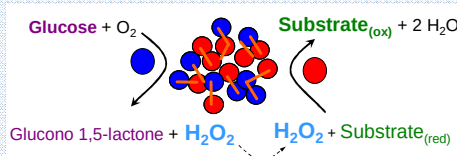


Fig. 1. Degradation of bisphenol A (BPA) in a continuous membrane reactor at lab-scale (activity of 1.3 KU/L, HRT of 111 min)

Approach 2: Combi-CLEAs for co-immobilization [3]

➤ **Biocatalysts:** Glucose oxidase from *A. niger* (●) and Versatile peroxidase from *B. adusta* (●)



➤ **Application:** Degradation of bisphenol A in a continuous membrane reactor

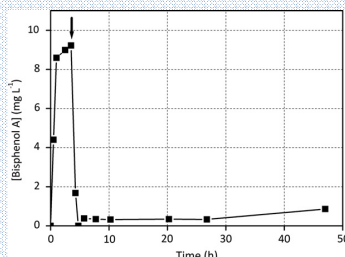


Fig. 2. Degradation of bisphenol A (BPA) in a continuous membrane reactor at lab-scale (VP activity of 25 U/L, HRT of 90 min)

Approach 3: Adsorption and cross-linking on functionalized (nano)particles [4]

➤ **Biocatalyst:** Laccase from *C. polyzona*

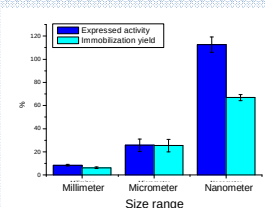
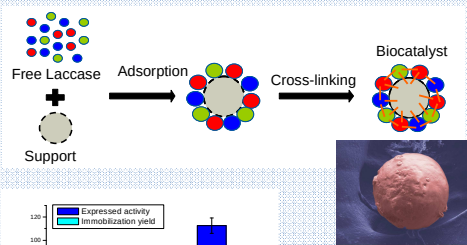


Fig. 3. Immobilization on silica particles (500 µm, 100 µm and 7 nm)

➤ **Application:** Degradation of mixtures of micropollutants

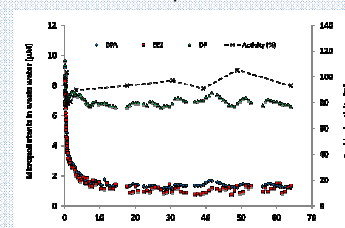


Fig. 4. Continuous treatment of the pollutants BPA, EE2 and DF in secondary wastewater effluent

Bio-inspired enzyme entrapment

➤ **Biocatalyst:** Oxidative and other types of enzymes and enzyme cascades

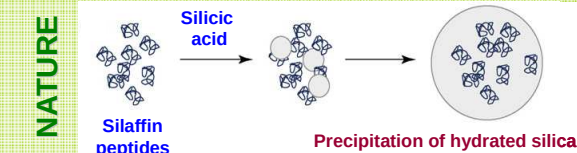


Fig. 5. SEM image of the silica cell walls of diatoms

Inorganic oxide precursors
Silica, Zirconia, Titania, Germania

Natural and synthetic templates
Silaffins, R5 peptide, Lysozyme, Silicateins, Poly-L-lysine, PEI, Spermine, spermidine

+ Enzyme

Entrapped enzyme in particles of different morphologies
Spherical particles (0.25-0.7 µm), Sheets, Ribbons, Nanofibers, Hexagonal platelets

➤ **Future applications:** Environmental and biocatalytic applications

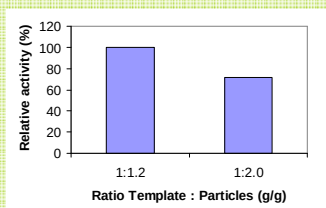


Fig. 6. Preliminary immobilization of laccase from *Thielavia* sp. by bio-inspired enzyme entrapment. The ratio between the synthetic template and the magnetic particles (used to allow an easy recovery) influences the final activity of the immobilized biocatalysts

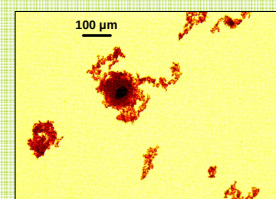


Fig. 7. Image of the immobilized biocatalysts obtained by optical microscopy

References

- [1] Cabana, H., Jones, J.P., Agathos, S.N. (2007) Journal of Biotechnology, 132, 23-31
- [2] Cabana, H., Jones, J.P., Agathos, S.N. (2008) Biotech. Bioeng., 102, 1582-1592
- [3] Taboada-Puig, R., Junghans, C., Demarche, P., et al. (2011) Bioresource Technol. 102, 6593-6599
- [4] Demarche, P., Junghans, C., Mazy, N., Agathos, S.N. (2012) New Biotechnol. 30, 96-103

Acknowledgements

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